EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Mechanism of freeze-induced protein destabilization	**Experiment number**: **LS-2601**
Beamline: ID-22	**Date of experiment**: from: to:	**Date of report**:
Shifts: 9	**Local contact(s)**: Andy Fitch	*Received at ESRF:*

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Report:

The primary objective of the study is to investigate solute/ice interaction using high-resolution X-ray diffraction. This data will be used to evaluate impact of solutes on ice recrystallization, and also get an insight into protein/ice interaction. Both of these topics are of primary interest for both applied (e.g., pharmaceutical science and technology) and fundamental science.

The main part of the experiment was devoted to aqueous solutions containing typical pharmaceutical solutes, including buffer (histidine), cryoprotector (sucrose), surfactant (polysorbate PS80), and four different proteins. The proteins are selected to cover a wide range of the isoelectric points, pI, from 4.7 to 11.35, and to represent different classes in terms of their applications. In particular, antifreeze protein (AFP), a monoclonal antibody (representing the most common type of protein drugs), lysozyme (the most common model protein), and recombinant humanized albumin are studied. Use of proteins of different pIs would allow us to evaluate role of electrostatics in protein/ice interaction. The X-ray diffraction patterns are collected at three sub-zero temperatures, i.e., 100 K, 228 K, and 258 K. Annealing is used to follow ice recrystallization as a function of time and temperature, and also to monitor amorphous/crystalline structure of solutes, in particular the buffer.

The list of samples tested is given in Table 1, along with the information of the number of scans performed and thermal conditions. In order to perform the initial data analysis, the background subtraction (using the data obtained for empty capillaries) has been assessed. For the background subtraction, the corresponding empty capillary pattern is subtracted from a particular dataset, e.g., "empty1" run 1 at 100K is subtracted from water 100K run 1, empty1 100K run 2 is subtracted from water 100K run2, etc. Examples of patterns with and without background subtraction are shown in Fig. 1. Considering that the background's contribution to the diffraction intensities is minor, further data analysis is performed without subtraction of the background scattering.

Examples of the patterns for water and AFP samples at 100K are provided in Fig 2. In the experimental ice pattern, all hexagonal ice (Ih) peaks are observed, while their relative intensities are significantly different from those in the theoretical pattern. The difference between theoretical and experimental intensities is probably due to randomly-enhanced growth of particular crystal faces. The relative intensities of the peaks in the presence of AFP are much closer to the theoretical intensities than those in pure water. However, several peaks in all three AFP samples have consistently lower intensites, as compared to the theoretical pattern of Ih, in particular 002 and 110, 200, 105 peaks.

X-ray diffraction patterns for pure water and AFP solutions at 228K are presented in Fig 3. There is no significant difference between 1st and 11th scans for water, while certain changes in the relative peak intesitites for AFP samples are noted. At 258K, some decrease in the peak intensities between run 1 and 11 is observed for water and AFP-water samples, but not AFP-histidine soluiton (Fig 4).

Examples of XRD patterns of solutions containing sucrose and two proteins are shown in Fig 5 and 6. All peaks of Ih are observed in these samples, although the relative intensities are different between different samples and are generally not the same as the theoretical intensities.

An important practical aspect of the study is an ability to accurately measure the local temperature on ice/solution interface. Taking into account that the sample temperature might not be exactly the same as the thermostat temperature (due to irradation-induced heating), the potential effect of the irradiation-induced heating is evaluated by measuring ice lattice parameters with and without full power, using an intensity attentuation factor of 5. In addition, patterns of pure ice, which are recorded at three different temperatures, will be used to evaluate the contribution of the thermal diffuse scattering form ice crystals to the scattering from the amorphous freeze-concentrated solution in proteni and sucrose samples.

Conclusion. The preliminary analysis of the data indicate that AFP inhibits growth of ice crystals in the direcitons corresponding to hkl 002, 110, 200, and 105. In addition, short-term annealing at 258K results in decreased intensity of diffraction peaks of Ih in water and water-AFP samples, which is unexpected and needs to be further evaluated. These observation should be considered as tentative, to be confirmed in a more systematic data analysis involving all 11 runs for each sample at different temperatures. Finally, contributions of the microstrain and crystallite size to the peak broadening will be eveluated using Williamson-Hall relationship.

Table 1. List of the samples studied and experimental conditions.

		100K		228K		258K	
#	**Sample description**	***Annealing: Yes / No***	***# of consecutive scans at this temperature***	***Annealing: Yes / No***	***# of consecutive scans at this temperature***	***Annealing: Yes / No***	***# of consecutive scans at this temperature***
1	Water	No	4	Yes	11	Yes	11
2	Water - 5X attenuated beam	No	4	No	4	No	4
3	5% w/v Sucrose in water	No	4	Yes	11	Yes	11
4	10% w/v Sucrose in water	No	4	Yes	11	Yes	11
5	20% w/v Sucrose in water	No	4	Yes	11	Yes	11
6	0.5 mg/mL AFP in water	No	4	Yes	11	Yes	11
7	1.0 mg/mL AFP in water	No	4	Yes	11	Yes	11
8	1.0 mg/mL AFP in 20 mM His buffer, pH 5.8	No	4	Yes	11	Yes	11
9	20 mM Histidine buffer + 5% w/v Sucrose, pH 5.8	No	4	No	4	No	4
10	20 mM Histidine buffer + 5% w/v Sucrose + 0.02% w/v PS80, pH 5.8	No	4	No	4	No	4
11	100 mg/mL IgG1 mAb + 20 mM Histidine buffer, pH 5.8	No	4	Yes	11	Yes	11
12	100 mg/mL IgG1 mAb + 20 mM Histidine buffer + 5% w/v Sucrose + 0.02% w/v PS80, pH 5.8	No	4	Yes	11	Yes	11
13	100 mg/mL rHA + 20 mM Histidine buffer, pH 5.8	No	4	Yes	11	Yes	11
14	100 mg/mL Lysozyme + 20 mM Histidine buffer, pH 5.8	No	4	Yes	11	Yes	11
15	Empty capillary-1	No	4	No	4	No	4
16	Empty capillary-2	No	0	No	0	No	4

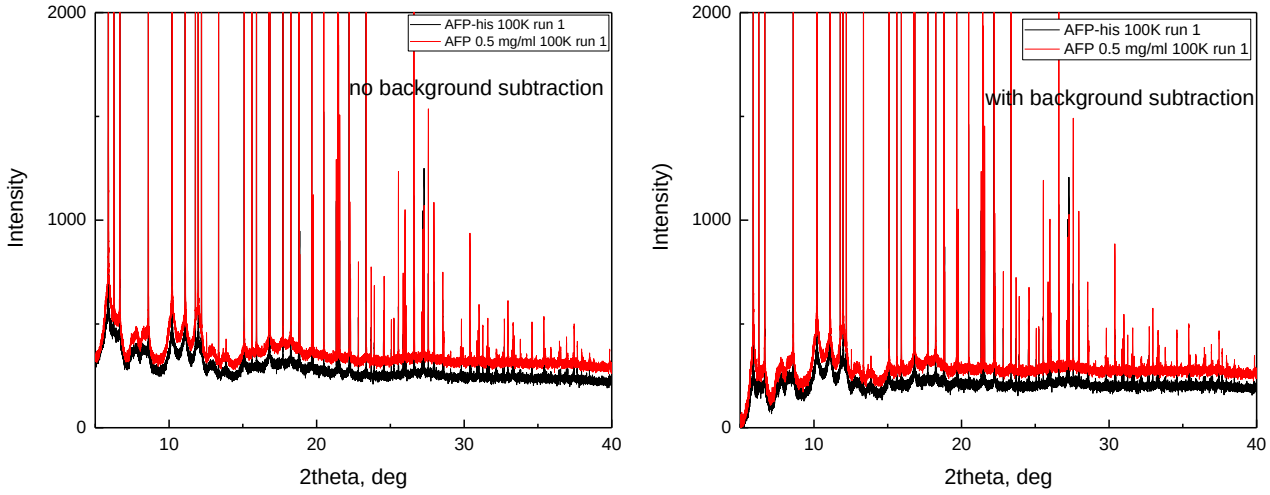

Figure 1. Examples of X-ray diffraction patterns with and without background subtraction at 100K

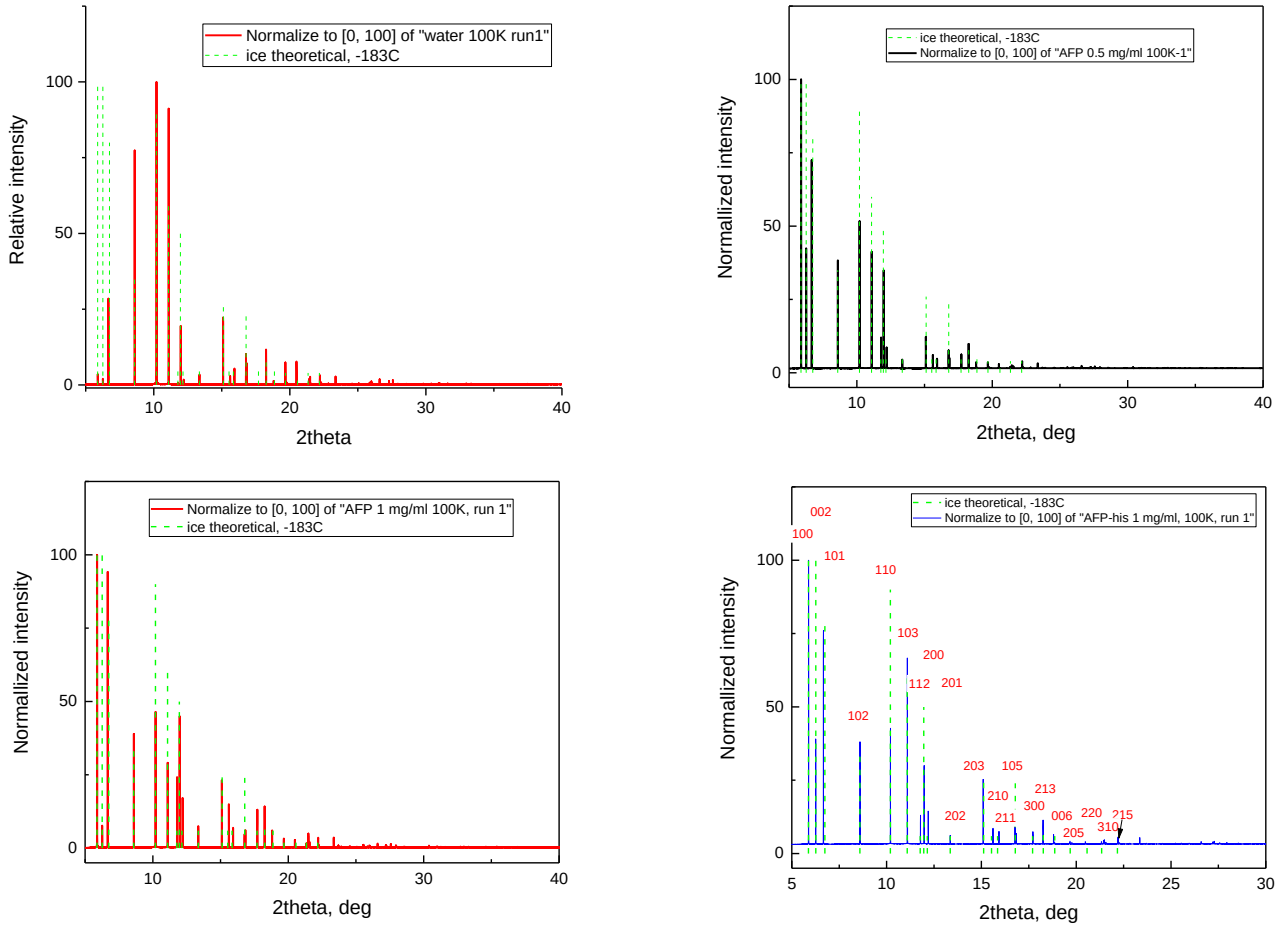

Fig. 2. X-ray diffraction patterns of pure water, AFP-water, and AFP-histidine samples at 100 K for first scans, with the background subtracted. Theoretical pattern for hexagonal ice at -183 °C is also shown.

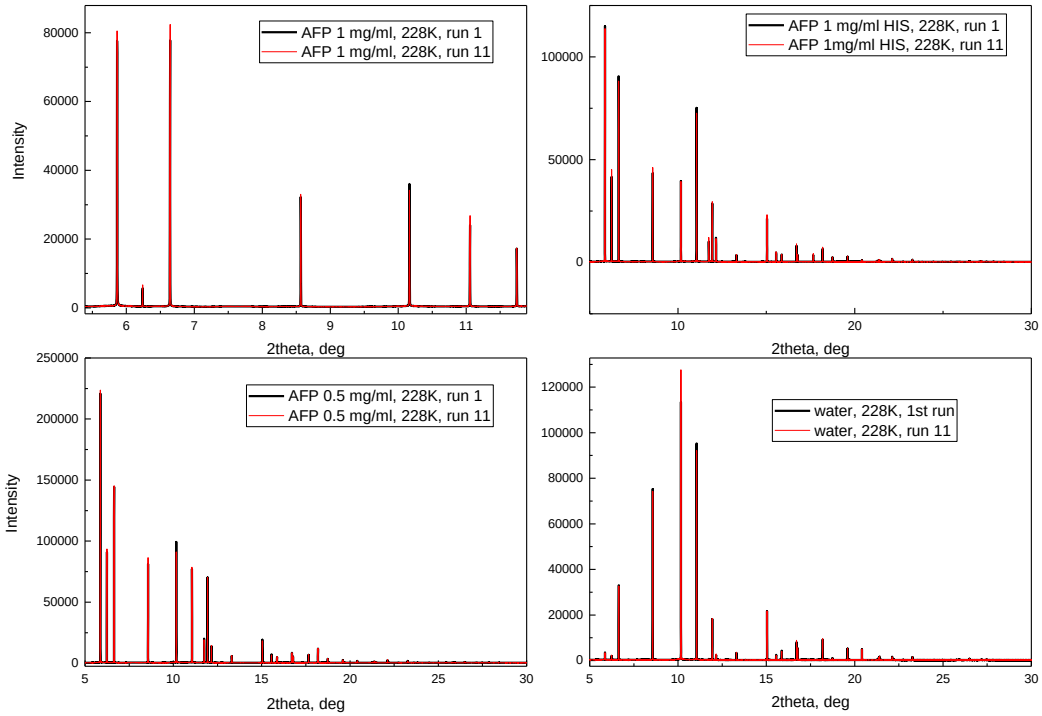

Fig. 3. X-ray diffraction patterns (no background subtraction) for AFP and pure water samples at 228K, run 1 and 11

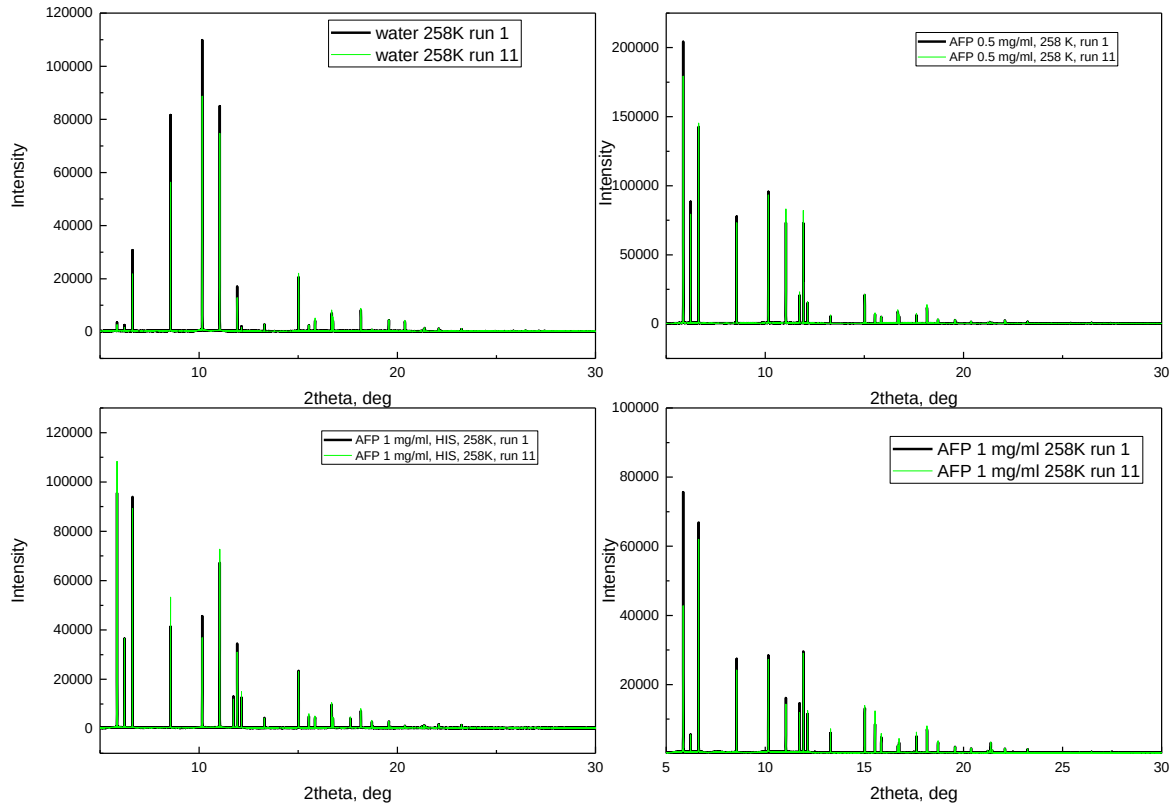

Fig. 4. X-ray diffraction patterns (no background subtraction) for AFP and pure water samples at 258K, run 1 and 11

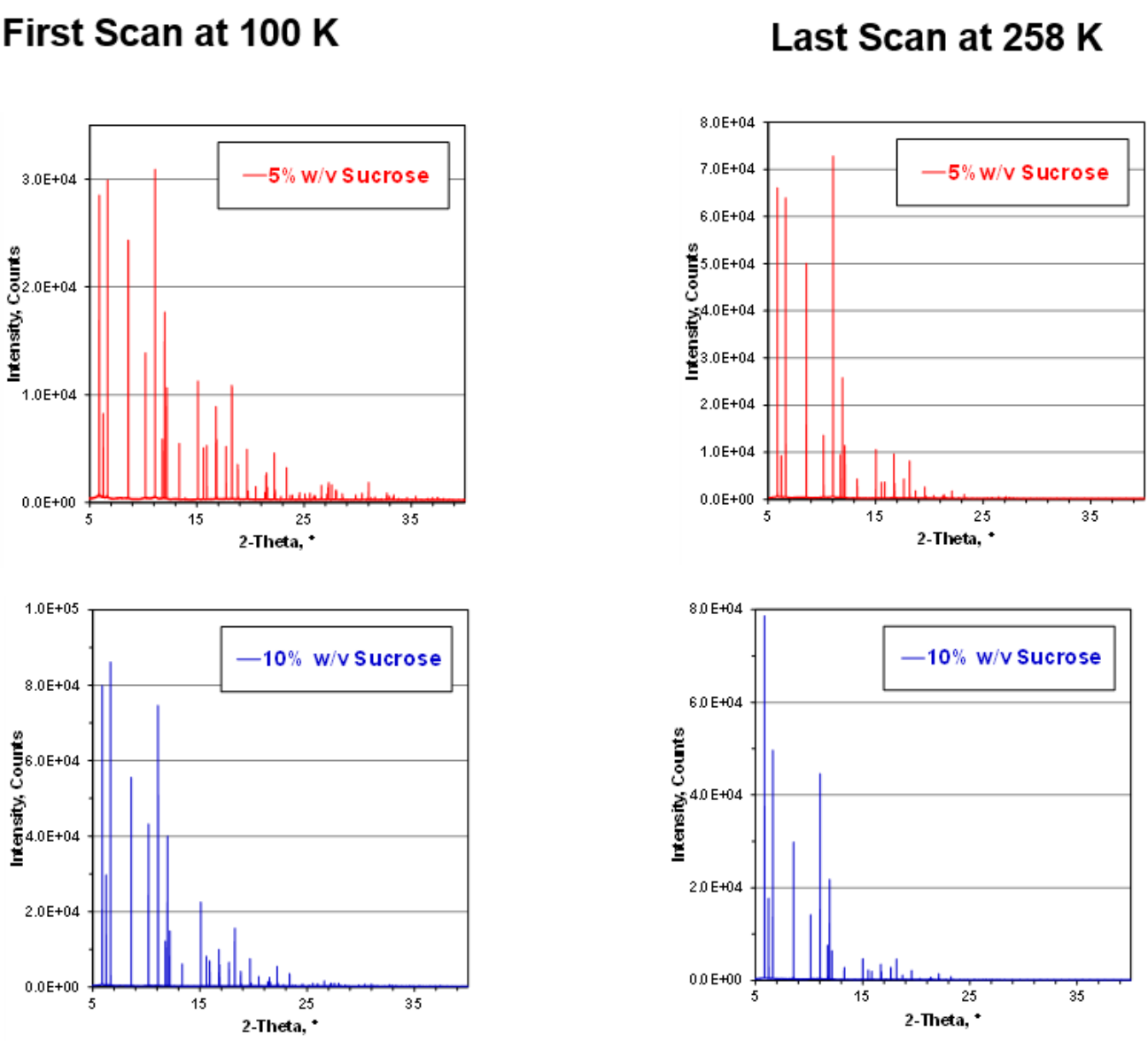

Figure 5. X-ray diffraction patterns sucrose solutions at 100 K (first scan; unannealed) and 258 K (last or 11th scan at 258K; annealed).

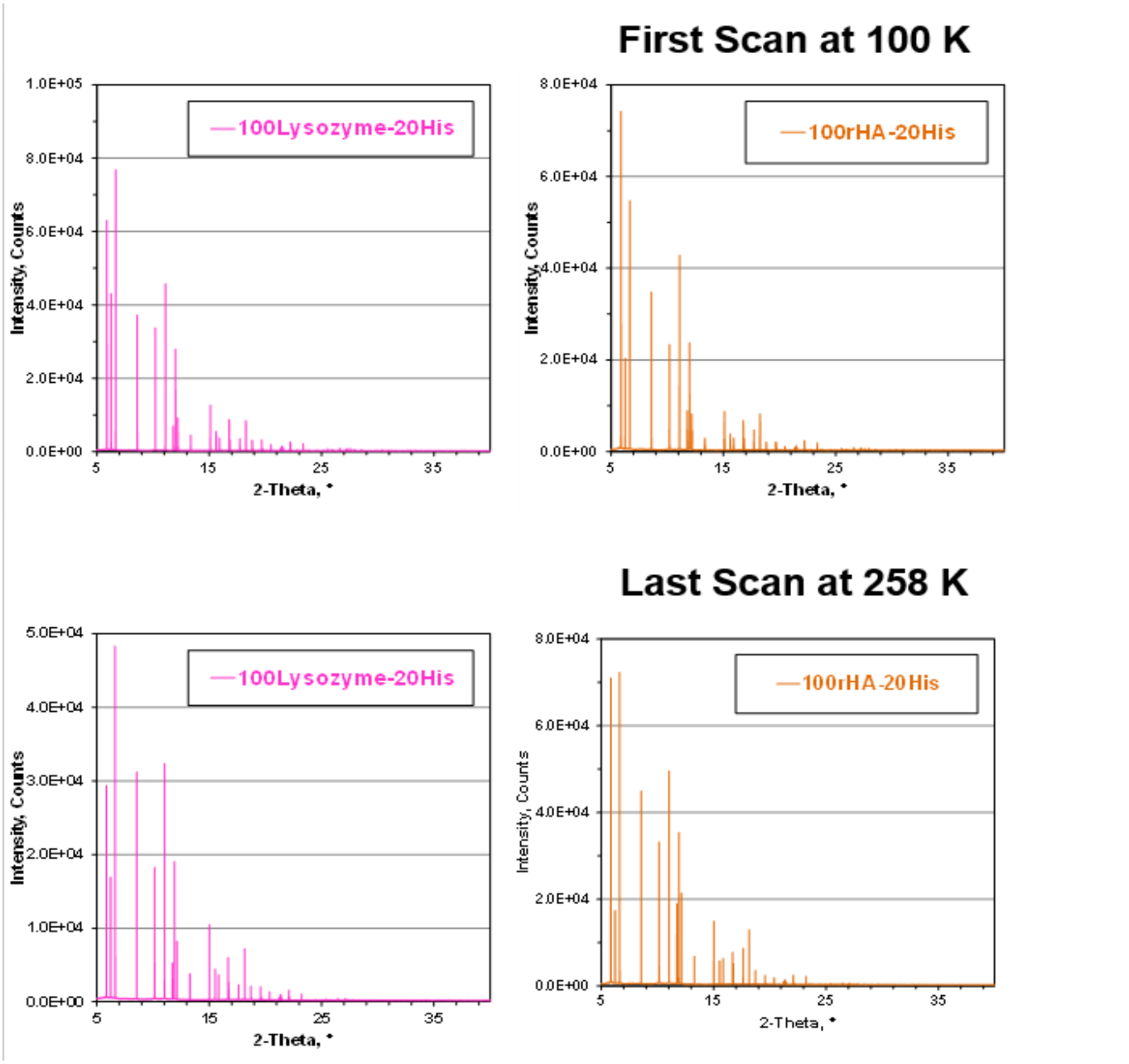

Figure 6. X-ray diffraction patterns for protein solutions at 100 K (first scan; unannealed) and 258 K (last or 11th scan at 258K; annealed).